

ELECTROKINETIC STUDIES OF MARINE OVA. VI. THE EFFECT OF SALTS ON THE ZETA POTENTIAL OF THE EGGS OF *STRONGYLOCENTROTUS PULCHERRIMUS*

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In the third paper of this series (Dan, 1936a), the zeta potential of the eggs of *Arbacia punctulata* was measured in NaCl and CaCl₂, both isotonic to sea water, and also in mixtures of the two in various ratios. From that study, the conclusion was drawn that the unfertilized surface of the *Arbacia* egg is ordinarily covered by a calcium compound of some sort which, however, dissolves away in the absence of calcium ions. This was based on three facts. (1) In higher calcium concentrations (more than 1/100 M of Ca in the isotonic mixture), the negative potential decreases, roughly corresponding to the calcium concentration, and this change is reversible. This is typical of the ionic effect of divalent cations and it shows that, within this range, the egg surface remains inert. (2) On the contrary, in lower calcium concentrations (less than 1/1000 M Ca), instead of the increase in negativity which is to be expected from the standpoint of the ionic effect, it decreases again and this decrease is irreversible. This is interpreted as due to the permanent loss of the calcium compound. (3) When *Arbacia* eggs are fragmented by centrifugal force into a lighter and a heavier part, the lighter part acquires a potential characteristic of a surface devoid of the calcium compound while the heavier part has a potential identical with that of the compound. This shows that the calcium compound forms a discrete membrane which ruptures on the fragmentation of the cell and is ultimately thrown down toward the heavy pole (Dan, 1936b).

In the first part of the present paper, the same sort of experiment is repeated with the present material and in the second part, the effect of cerium chloride is examined.

MATERIAL AND METHOD

Eggs of the sea-urchin, *Strongylocentrotus pulcherrimus*, were used throughout. Unless specified, the jelly was always removed before the cataphoretic measurements. The method of removing the jelly and the method of measuring the zeta potential are the same as were used in the preceding paper (Dan, 1947).

SODIUM-CALCIUM MIXTURES

The data are given in Table 1 and Figure 1. In the region of higher calcium concentrations, as in the case of *Arbacia*, the absolute magnitude of the negative potential decreases as the divalent calcium ions increase and this change in potential is perfectly reversible. The parallelism between the *Strongylocentrotus*-curve and the *Arbacia*-curve in this region is particularly striking (see Fig. 1). This means

that the potential change under consideration is a typical ionic effect due to calcium ions. On the contrary, the two curves diverge in the region of lower calcium concentrations. While the *Arbacia*-curve comes down in dilutions beyond 1/1000 M calcium in mixture, the *Strongylocentrotus*-curve goes sharply up, and this change is reversible.

TABLE 1

The zeta potentials of the unfertilized eggs of Strongylocentrotus pulcherrimus under various conditions. The values are given in millivolts with standard errors.

With jelly	-37.3±0.70
Without jelly	-33.9±0.50
Without jelly, 4 hours in NaCl, measured in sea water	-33.9±0.53
Jelly not removed, 3 hours in urea, measured in sea water	-32.9±0.60
Jelly removed by acid, 4 hours in urea, measured in sea water	-33.0±0.50
Heat-killed in NaCl	-26.8±0.44
1/2 M NaCl (intact cells)	-39.7±0.67
NaCl + 1/100 M CaCl ₂	-33.4±0.64
NaCl + 1/10 M CaCl ₂	-28.9±0.63
NaCl + 1/5 M CaCl ₂	-22.7±0.67
1/3 M CaCl ₂	-17.5±0.40

If the line of argument used for the case of *Arbacia* eggs is pursued further, the above result must be interpreted as indicating that either the *Strongylocentrotus* eggs do not react with calcium ions or the covering layer of the calcium compound of this species is not dissolved away in pure NaCl. The author inclines to adopt the latter view. To some extent, this is supported by the fact that *Strongylocentrotus* eggs are more resistant to cytolysis in NaCl than other species. Of course, this result should not be taken to mean that the covering layer of *Strongylocentrotus* eggs cannot be dissolved at all under any circumstance. The peptization of this compound may occur in some other solutions or even in 1/2 M NaCl if a different treatment is used. In the present experiment, for the change of medium, the following procedure was selected as standard. To 20 cc. of the egg suspension in a beaker, 200 cc. of 1/2 M NaCl was gently added and the suspension gently stirred to insure complete mixing. After the eggs had settled, as much as possible of the supernatant solution was siphoned off and fresh NaCl solution was gently added and stirred as before. This washing process was repeated from four to five times. Consequently, the eggs were in the NaCl solution for 30 to 40 minutes before measurement began.

In order to test other possibilities, in one series the time factor was changed. After the usual washings, the eggs were left in NaCl so that the total sojourn of the eggs in this solution was 4 hours. In spite of this the potential did not change (Table 1). In another series, isotonic urea solution was tried and this series was further divided into two groups. In one group, unfertilized eggs with jelly were put directly into urea and left there for 3 hours. Needless to say, the jelly was dissolved by the time cataphoretic measurement was begun. In the second group, the jelly was removed by acid and the eggs were left in urea for 4 hours. Still, no change was observed in the potential. The potential was found to change only after the eggs were killed by heat in NaCl. This is undoubtedly due to the effect of the internal granules (see Dan, 1933). Therefore the above result shows that

the surface calcium compound of *Strongylocentrotus* (if there is such a compound) is much more stable than that of *Arbacia* eggs. Unfortunately the number of washings was not changed. At any rate, a definite conclusion can be drawn that the egg surface of this species is quite resistant to the absence of calcium ions and, under the experimental conditions here dealt with, it can be looked upon as an inert surface through the entire range of calcium concentrations.

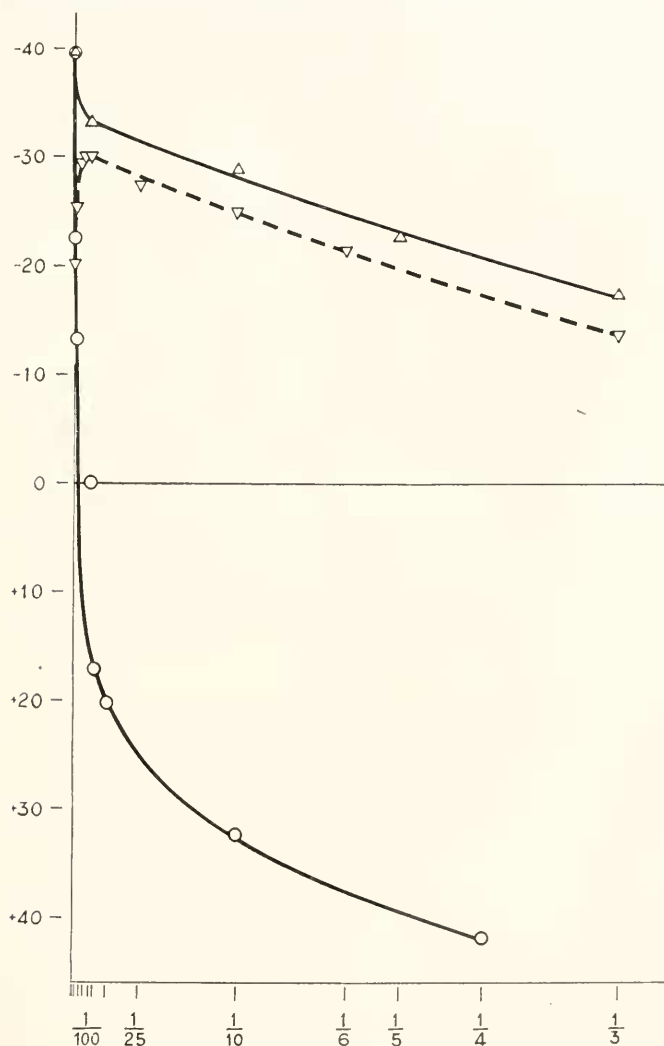


FIGURE 1. The change in the zeta potentials of the unfertilized eggs of *Strongylocentrotus* in mixtures of isotonic NaCl and CaCl₂ (Δ-Δ) and of NaCl and CeCl₃ (O-O). The ratios of the mixtures are expressed by the Ca- and Ce-concentrations respectively which are taken as the abscissae. The ordinates are the potential values. The broken line is the curve for *Arbacia* eggs in Na-Ca mixtures (▽-▽). Note the parallelism between the *Arbacia* curve and the *Strongylocentrotus* curve in higher Ca-concentrations and their divergence in the region of low Ca content.

SODIUM-CERIUM MIXTURE

After examining the effect of calcium, cerium was studied as a representative of the polyvalent ions. The egg jelly was removed and the solutions were changed in the same way as in the calcium series. One-half M NaCl and $\frac{1}{4}$ M CeCl_3 were used either singly or in mixtures. For this purpose, the fact that the surface of *Strongylocentrotus* eggs remains unaffected in pure NaCl is of a great advantage, because it is impossible to mix the cerium solution with sea water on account of the formation of precipitates.

As far as the present material is concerned, the immersion of the eggs in cerium solution is not very harmful as long as the time of sojourn is not too long. On insemination of such eggs after having been returned to sea water, they are fertilizable except for the fact that the fertilization membrane fails to separate from the egg surface (tight membrane eggs).

On the other hand, there are two complications of a physicochemical nature involved in the use of cerium salts. One is the contamination of ceric ions in a preparation of cerous salt. Trivalent cerous ion is rather susceptible to oxidation and it spontaneously changes into tetravalent ceric ion. The former is colorless but the latter is light brown, and this transformation is clearly perceptible if the cerous solution is left in a transparent glass bottle. Unfortunately, this change seems to occur even in the crystals of the former as was revealed by a slight brownish tint of the cerous chloride crystals used by the author. But in the current study, no attempt was made either to obtain colorless crystals or to remove ceric ions, since it was considered that the maintenance of a constant proportion of the two ions would be sufficient for the present purpose. However, an attempt was made to know the ratio of the two by titration after Knorre's method (Knorre, 1897). Ceric ions, 0.0007 M, were found in 0.25 M solution by weight of the crystals; i.e., the ratio was 1:357. As a result, in the following paragraphs, the rather loose term "cerium solution" is used instead of specifying the condition precisely.

TABLE 2

*The hydrogen ion concentrations of various mixtures of 1/2 M NaCl and 1/4 M CeCl_3 .
The ratio of a mixture is expressed in Ce concentration after mixing.*

Concentrations (M) of Ce in NaCl	1/3000	1/1000	1/100	1/80	1/50	1/4
pH	7.0	6.8	6.2	5.7	5.6	4.7

The other complication is the change in hydrogen ion concentration of cerium solutions. As is well known, among polyvalent cations, it is very difficult to prepare solutions of different concentrations with the same pH, because the ions precipitate. In spite of this, measurements were carried out for a reason which will be explained below. The pH's of the cerium solutions used are given in Table 2.

RESULTS

The result of cataphoretic measurements is given in Table 3 and graphically represented in Figure 1. As is evident, the curve is again typical of an adsorption isotherm, except for the potential value in $\frac{1}{100}$ M cerium which is obviously off.

In this connection, it must be pointed out that sea-urchin eggs, like any colloidal particles, stick together when the surface potential is abolished. In a $\frac{1}{100}$ M solution the eggs are extremely adhesive. (This point is specially investigated in a following paper.) On account of this, it is quite difficult to take accurate readings. As mentioned above, many eggs clump together in this medium and when large lumps settle, they cause turbulence in the surrounding solution which, in turn, affects the readings. The fact that only a few eggs remain single adds to the difficulty. For such reasons, it seems to be better to determine the iso-electric concentration of cerium solution from the smoothed curve such as is shown in Figure 1.

TABLE 3

The zeta potentials of the unfertilized eggs of Strongylocentrotus pulcherrimus (without jelly) in various mixtures of 1/2 M NaCl and 1/4 M CeCl₃, the ratios of the mixtures being expressed in Ce concentrations in the resulting mixtures. The values are given in millivolts with standard errors.

1/2 M NaCl	-39.7±0.67
NaCl + 1/3000 M CeCl ₃	-22.7±0.58
NaCl + 1/1000 M CeCl ₃	-13.3±0.57
NaCl + 1/100 M CeCl ₃	- 0.4±0.80
NaCl + 1/80 M CeCl ₃	+17.2±0.49
NaCl + 1/50 M CeCl ₃	+20.3±0.54
NaCl + 1/10 M CeCl ₃	+32.3±0.88
1/4 M CeCl ₃	+41.8±0.80

DISCUSSION

The result reported in the first section of this paper led the author to the conclusion that, contrary to the case of *Arbacia*, the surface of the *Strongylocentrotus* eggs remains inert irrespective of whether calcium ions are present or absent in the surrounding medium. However, this statement signifies at the same time that the change in the zeta potential observed must be brought about by the adsorption of the calcium or cerium ions. In fact, the courses of both calcium- and cerium-curves are quite like any adsorption isotherms, but this situation can be analyzed further.

It is generally understood that the effects of ions on the zeta potential are two-fold. One is the effect of ions on the thickness of the diffuse layer (in Gouy's sense) and the other is the adsorption of ions to the surface (Müller, 1933). Fortunately, in such concentrated media as used here, the first proposition is practically negligible and it is permissible to consider solely the second proposition. The next point is that the solutions studied here are not solutions of a single salt but mixtures of two salts. In the Na-Ca series, as the calcium concentration is raised, successively three ions of sodium are replaced by two ions of calcium, and in the Na-Ce series, two ions of sodium are exchanged for one ion of cerium. Besides this, in the cerium series, the hydrogen ion concentration is also changed. But Rona and Michaelis (1919), after studying the adsorption of binary mixtures of salts by charcoal, came to the conclusion that when two salts are present, the more adsorbable ions drive the less adsorbable ions out of the surface so that the net result is very much like the adsorption of a single salt. This simplified picture may be applicable for the present case, since the adsorbability of trivalent cations far exceeds that of monovalent ions for a negatively charged surface.

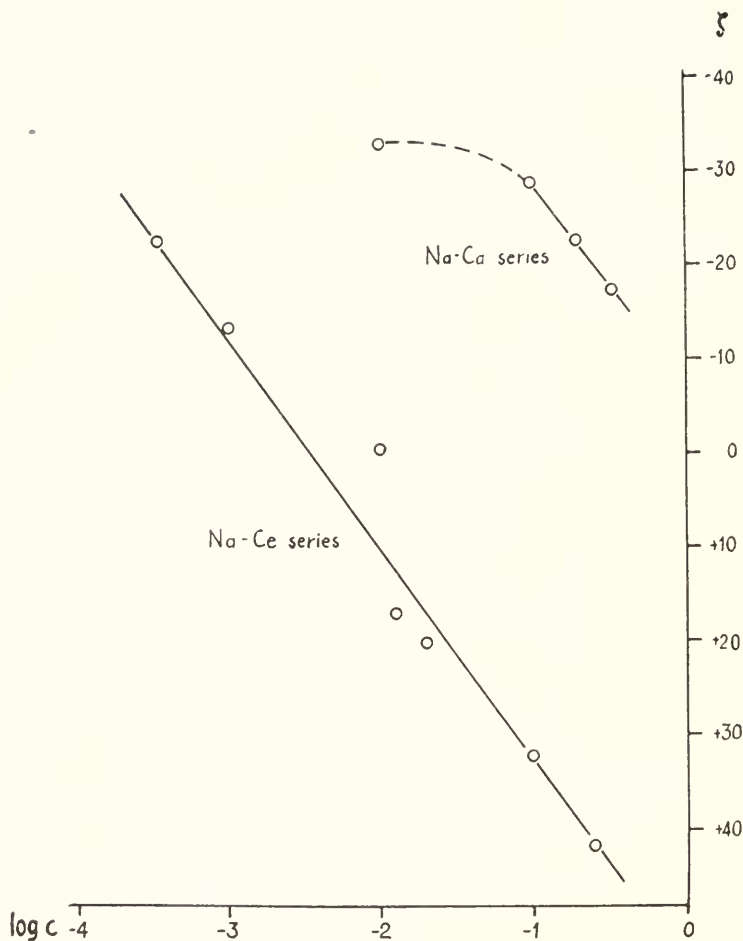


FIGURE 2. The plotting of the potential values against the logarithm of the concentrations. The Ce-series makes a straight line. The Ca-series does so only in the high Ca range.

Allowing this much simplification, the data can be checked by a formula for adsorption. Freundlich's adsorption isotherm will be used for testing, which is

$$a = \alpha \cdot c^{1/n},$$

where a is the quantity adsorbed, c , the concentration, α and n are constants (see Freundlich, 1930, p. 244). As long as this formula holds, plotting of $\log a$ against $\log c$ ought to give a straight line. For electrokinetics, instead of a , the charge density σ must be used. But since σ is a function of $e\zeta$, in place of $\log \sigma$, the potential value ζ can be directly plotted (see also Müller, 1933).*

* $\sigma = \pm \sqrt{\frac{DKI}{2\pi} \sum n^0 (e^{-2e\zeta/KT} - 1)}$, where σ is the charge per cm^2 ; D , the dielectric constant;

K , the Boltzmann's constant, namely, $R/N = 1.37 \times 10^{-16}$; T , the absolute temperature; n^0 , the number per cm^3 , of ions of valency Z far removed from the surface. In other words, n^0 is connected to the molar concentration c with the relation of $n^0 = c \cdot 606 \times 10^{-20}$. e is the base of natural logarithms; e , the electronic charge.

The curve thus plotted is shown in Figure 2. As is evident, the cerium series makes a straight line (neglecting the value in $1/100$ M). This can be taken as a proof that the change in potential in cerium solutions is due to the adsorption of the cerium cations. A similar plotting for the calcium series is also shown in Figure 2. In this case, although a straight line is obtained for the higher calcium region, it deviates in the lower concentrations. This can probably be interpreted as indicating that in the low calcium region, the effect of sodium ions is no longer negligible because the difference in adsorbability and in the potency in influencing the zeta potential between sodium and calcium is not so great as between sodium and cerium.

The next point of importance is the agglutination of the eggs under the isoelectric condition. However, since this problem will be taken up separately in a following paper, the discussion will be deferred until then.

SUMMARY

1. The electrokinetic potentials of the unfertilized eggs of *Strongylocentrotus pulcherrimus* were measured in mixtures of various ratios of isotonic NaCl and CaCl_2 and of NaCl and CeCl_3 .

2. In the Na-Ca series, as the concentration of Ca-ions in the mixture was raised, the absolute magnitude of the negative potential of the eggs decreased. In the Na-Ce series, with the increase in concentration of Ce-ions, the sign of the zeta potential was reversed.

3. At the isoelectric point, the eggs agglutinated.

4. The course of the potential change can be attributed to the adsorption of the cations.

5. The existence of a covering layer and its dissolution in the absence of Ca-ions such as were seen in *Arbacia* eggs have not been found in *Strongylocentrotus* eggs.

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